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Quantum Yield for the Photoproduced Electron Paramagnetic Resonance Signal in Chromatophores from *Rhodospirillum rubrum**

P. A. Loach and K. Walsh

ABSTRACT: A 1:1 correspondence has been demonstrated between the concentrations of photoproduced spins in chromatophores of *Rhodospirillum rubrum*, as measured by electron paramagnetic resonance techniques, and the concentration of the reaction center primary electron donor molecule (P_{870} or $P_{0.44}$), as measured by absorbance change at 865 nm. In addition a significantly high quantum yield (0.8 with 880-nm light) has been determined for the electron paramagnetic resonance signal production. When all existing data relating to the photoproduced absorbance change at 865 nm and the photoproduced electron paramagnetic resonance signal are reviewed, together with these results, the conclusion seems inescapable that these are two properties reflecting change in oxidation state of a single molecular species.

From the very first measurements of the photoproduced electron paramagnetic resonance signal in photosynthetic systems (Commoner *et al.*, 1956, 1957; Sogo *et al.*, 1957) it was suggested that the signal arises as a result of photoexcitation of a chlorophyll (or bacteriochlorophyll) molecule at the reaction center. Subsequent experiments in many different laboratories have shown that (a) the location and shape of the signal is consistent with its identification as a porphyrin derivative (Calvin, 1959; Mauzerall, 1968; Mauzerall and Feher, 1964; Mauzerall *et al.*, 1967); (b) the ability to form it at low temperature (Androes *et al.*, 1962) is consistent with its assignment to a primary event; (c) its redox midpoint potential (Loach *et al.*, 1963) and kinetics

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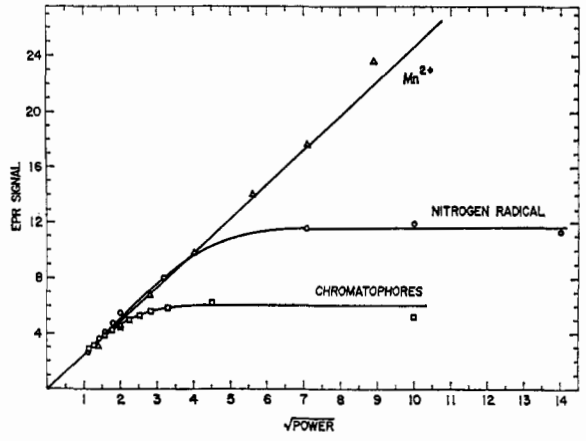

FIGURE 1: Variation of electron paramagnetic resonance signal magnitude with microwave power. (△) Points for a solution of 1×10^{-4} M $Mn(C_2H_3O_2)_2$ in 0.1 N HCl. (○) Points for 6×10^{-6} M 2,2,5,5-tetramethyl-3-carboxypyrroline-*N*-oxyl in 0.1 M glycylglycine buffer at pH 7.5. (□) Points for a suspension of chromatophores prepared from *R. rubrum* in 0.1 M glycylglycine buffer at pH 7.5, 0.01 M $K_4Fe(CN)_6$; A_{880} = 100 in a 1-cm cuvet, saturating light intensity, *T* 25°. Modulation amplitude was 0.1 G for the nitrogen radical, 0.5 G for chromatophores, and 5 G for $Mn(C_2H_3O_2)_2$. The normalizing factor for the ordinate was chosen so that the initial slopes were identical.

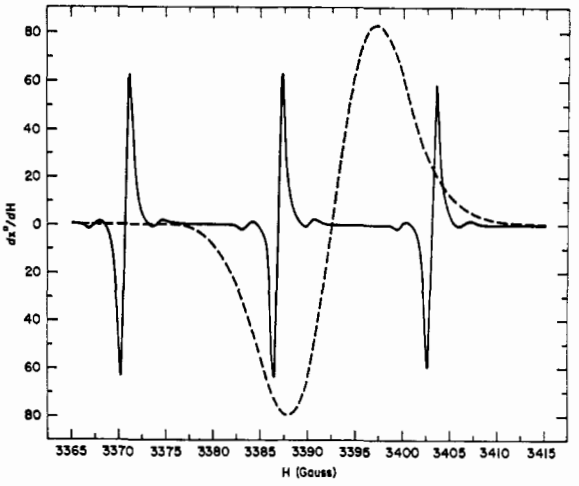

FIGURE 2: Light-induced electron paramagnetic resonance signal of chromatophores from *R. rubrum* (dashed curve) compared with the electron paramagnetic resonance signal of 3.2×10^{-4} M 2,2,5,5-tetramethyl-3-carboxypyrroline-*N*-oxyl (solid curve). Both samples were in 0.1 M glycylglycine buffer, pH 7.5, *T* 25°. A_{880} for chromatophores in a 1-cm cuvet = 100, saturating light intensity. Modulation amplitude = 0.5 G for chromatophores and 0.1 G for the nitrogen radical. Microwave power was 2 mW for both. The *g* value determined for chromatophores, by comparison with peroxylamine disulfonate as a standard material (assumed *g* value = 2.0057 with 13.0 G splitting in H_2O), is 2.0027.

of decay (Loach and Sekura, 1967) have been matched, under a variety of conditions, with those for photoproduced absorbance changes at 865 nm in chromatophores of *Rhodospirillum rubrum*.

The first attempt, by Beinert and Kok (1964), to demonstrate the quantitative relationship between the electron paramagnetic resonance signal size and the amount of electron donor present according to the magnitude of absorbance change, suggested that either a significant inconsistency exists between the two, or that quantitative determination of the electron paramagnetic resonance change was not achieved. Their study was primarily concerned with green plant and algae systems. The present data are relevant to bacterial systems where it will be shown that an excellent correlation exists between the concentration of spins and the concentration of $P_{0.44}$ as determined from change in absorbance.

With the confidence that the number of spins can be accurately measured, we have extended the measurements to include determination of the intensity of light absorbed. Thus, we have determined the quantum yield for spin production in chromatophore systems and compared the result with earlier measurements of the quantum yield for $P_{0.44}$ oxidation (Loach and Sekura, 1968).[1]

Experimental Section

Chromatophore fractions from *R. rubrum* and *Rhodopseudomonas spheroides* were prepared as previously described (Loach *et al.*, 1963). The material was resuspended in 0.10 M glyclyglycine buffer (pH 7.8) and stored at 5°. The absorbance of this material at 880 (*R. rubrum*) or 850 nm (*R. spheroides*), for a 1-cm path length, was usually about 600. Samples used for electron paramagnetic resonance measurements were diluted with distilled water to an absorbance of 100 or about 5 depending upon the experiment.

A Varian E-3 spectrometer and aqueous sample holder were used for all measurements. Each time the sample holder was placed in the electron paramagnetic resonance spectrometer care was taken to have the same flat surface forward and the tube held at the same verticle height. A precision of ±1% could be routinely obtained in multiple measurements of the same sample.

The manganese acetate and manganese chloride used for standard samples were Baker Analyzed reagents. They were freshly dissolved in 0.1 N HCl when used. The 2,2,5,5-tetramethyl-3-carboxypyrroline-*N*-oxyl was a gift[2] whose preparation has been described (Rozantsev and Neiman, 1964; Krinitskaya, 1965).

For quantitative comparison of electron paramagnetic resonance signals the following must be carefully considered. (1) Power saturation of the standard material and of the unknown must be determined so that a setting of power sufficiently far from saturation of either sample may be selected for the comparison measurement. Variation with power of the electron paramagnetic resonance signals of the two standard systems and *R. rubrum* chromatophores are shown in Figure 1. (2) The modulation amplitude used has to be significantly less than the half-width of the narrowest line so

[1] A preliminary report of the results contained herein was given at the 5th International Congress on Photobiology, Aug. 26–30, 1968, at Dartmouth, N. H.

[2] We would like to express our sincere thanks to Professor Brian Hoffman for providing us with this material.

TABLE I: Comparison of Line Shapes of Electron Paramagnetic Resonance Signals.[a]

Height (% of max)	Theoretical		Experimental		
	Guassian	Lorentzian	Mn^{2+}	Nitrogen Radical	Chromatophores
100	*a*	*a*	*a*	*a*	*a*
80	1.5*a*	1.65*a*	1.69*a*	1.43*a*	1.45*a*
60	1.8*a*	2.10*a*	2.03*a*	1.77*a*	1.76*a*
40	2.1*a*	2.75*a*	2.64*a*	2.04*a*	2.10*a*
20	2.5*a*	3.80*a*	3.55*a*	2.28*a*	2.37*a*
10	2.8*a*	5.0*a*	5.00*a*	2.86*a*	2.62*a*

[a] Values for the theoretical Gaussian and Lorentzian shapes were taken from the Varian EPR Operational Techniques Publication No. 87-114-402, pp 5–21. *a* is equal to one-half the distance from peak to peak along the magnetic field coordinate. Modulation amplitude was 0.1 G for the chromatophores and the nitrogen radical, and 5 G for $Mn(C_2H_3O_2)_2$. The microwave power was 2 mW in all cases. $t = 25°$. The chromatophores and the nitrogen radical were in 0.10 M glycylglycine buffer (pH 7.5). $Mn(C_2H_3O_2)_2$ was in 0.1 N HCl.

that peak height distortion does not occur. (3) The shapes of the signals being compared should be identical (Figure 2 and Table I) for the most accurate results. The following relationship can be used to relate the two signal concentrations (Poole, 1967)

$$\frac{N^A}{N^B} = \frac{(D^A)(g^B)^2 S^B(S^B + 1)(\Delta H^A)^2}{(D^B)(g^A)^2 S^A(S^A + 1)(\Delta H^B)^2} \times \frac{(\Lambda'^A)(H^B{}_{mod})(y'^A)(G^B)}{(\Lambda'^B)(H^A{}_{mod})(y'^B)(G^A)}$$

where the superscript A refers to the standard system and B to the unknown system; N is the number of spins present; D is the factor needed to change the area of the strongest line into the area of an unsplit signal; G is the g value; S is the spin quantum number; ΔH is the peak to peak line width; Λ' is the shape factor which is 1.03 for a Lorentzian shape and 3.63 for a Gaussian shape; H_{mod} is the modulation amplitude setting of the instrument; y' is the peak to peak height of the strongest line; and G is the electronic gain of the instrument. Since the unknown system is measured under the same instrumental conditions as is the standard, many of the terms in the above equation can be neglected.

The curve shapes of the three signals measured are compared in Table I with those expected for Gaussian and Lorentzian shapes. It may be seen that the 2,2,5,5-tetramethyl-3-carboxypyrroline-*N*-oxyl signal and the chromatophore signal are good approximations of a Gaussian shape while the manganese signal approximates a Lorentzian shape. Determination of the concentration of Mn^{2+} by electron paramagnetic resonance, using the nitrogen radical as a reference standard, gave results that agreed to within 5% with the known molarity.

Since one of the electron paramagnetic resonance signals being compared is light produced care must be taken to ensure that sufficient light intensity for saturation is absorbed by all portions of the sample volume which lie in the sensitive portion of the microwave cavity. This means that the absorbance of the sample must be low enough to permit the light to easily reach the distant side of the glass holder and that the angle of the light imposed on the screen in front of the cell must be sufficiently varied so that light arrives at all parts of the sample holder. The use of too much light intensity must also be guarded against because additional bacteriochlorophyll radicals can be produced from pigments other than the reaction center bacteriochlorophyll. For the concentration comparison with manganese as the relevant standard system it was possible to use lower concentrations of chromatophores because higher settings of modulation amplitude could be used. Many systems had an absorbance at 880 nm near 5.0 in a 1-cm cuvet. Figure 3 shows the light saturation profile for such a system.

For quantum yield measurements the amount of light absorbed by the samples had to be determined accurately. For these experiments a parallel beam of light was produced so that its cross-sectional area was uniform. The exciting light was that from a 1000-W tungsten projection lamp, the voltage to which was controlled with a Variac. The authenticity of the optical arrangement was checked by measuring the quantum yield for the ferrioxalate actinometer system (at 0.15 M) in the aqueous sample holder without the electron paramagnetic resonance screen in front of it. A value of 1.30 was obtained in excellent agreement with our previous results with this system (Loach and Sekura, 1968) and in fair agreement with the literature value of 1.22 for these particular conditions (Hatchard and Parker, 1956). This actinometer solution was then used with the screen in place in order to determine the actual energy reaching the sample. From this measurement, 41% of the light incident upon the screen passed through it. The amount of light passing through the screen was also calculated by measuring the fraction of open area from a photograph of it. The value obtained from this measurement was 39%. An average value (40%) was

TABLE II: Concentration of $P_{0.44}$ in Chromatophores.[a]

Type of Measurement	*R. rubrum* (μM/A_{880})	*R. spheroides* (μM/A_{850})
A_{865} (ϵ_{mM} 90)	0.28	
A_{880} (ϵ_{mM} 90)		0.15
Electron paramagnetic resonance[a]	0.25	0.16

[a] Microwave power = 2 mW, modulation amplitude = 0.1 G. Sample optical density in 1-cm cuvet = 5.0. Reference standard was 2,2,5,5-tetramethyl-3-carboxypyrroline-*N*-oxyl. T = 25°; 0.10 M glycylglycine buffer (pH 7.5).

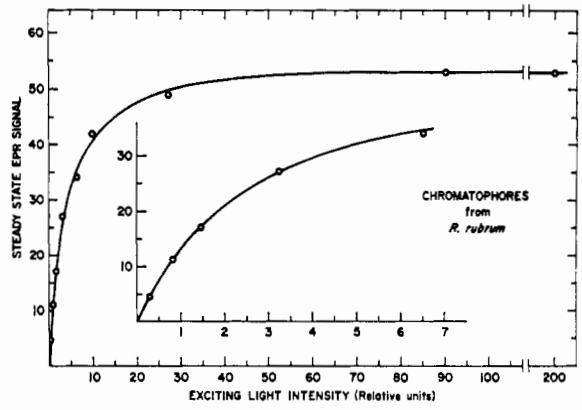

FIGURE 3: Variation with light intensity of the steady-state electron paramagnetic resonance signal in chromatophores from *R. rubrum*. A_{880} = 6.5 in a 1-cm cuvet, 0.05 M phosphate buffer, pH 7.5; 0.01 M $K_4Fe(CN)_6$. The relative intensity of the light was measured using a YSI Model 65 Radiometer placed in the beam of light originating from a 1000-W tungsten projection lamp. This measurement was made prior to the beam's passage through one Corning 7-69 color filter, two Corning 7-56 color filters, and then onto the sample. Modulation amplitude = 10 G. Microwave power = 2 mW.

used for the experiments reported. Two thermopiles, which have been previously described (Loach and Sekura, 1968), were used to measure energy reaching the sample holder at 365 nm (passing through a 5-cm copper sulfate solution and a Corning 7-39 color filter) or at 880 nm (passing through a Baird Atomic B-9 narrow band filter and a 5-cm water filter). From these data it was then possible to calculate the light energy absorbed by the biological sample under a variety of intensities and excitation areas. The concentration of chromatophores used for all quantum yield determinations was high enough (absorbance at 880 nm equal to 100 in a 1-cm cuvet) so that less than 2% of the light entering the sample escaped from it.

From the quantitative comparison with a standard system, the change in the number of spins *vs.* time could be directly measured. Since not all parts of the aqueous cell contents are detected with the same sensitivity, the excitation beam was systematically made smaller (by masking off outer portions) while keeping it centered in the middle of the cell. This process of using a smaller and smaller exciting beam was continued until the apparent quantum yield calculated no longer reflected a change in the area being excited.

For much of our previous electron paramagnetic resonance data we knew the molarity of the sample but not the actual number of spins detected. In order to interconvert between concentration and the total number of spins measured an instrumental constant was determined which may be referred to as the effective volume of the aqueous cell. Using a procedure previously employed by Hyde[3] the effective volume of our aqueous cell was found to be 0.035 ml. The method of determining this latter value uses a standard solution of a stable radical (the sample used was 2,2,6,6-tetramethylpiperidine-*N*-oxyl) in a solvent with low dielectric constant, such as benzene. Two measurements were made. For one of these, 0.010 or 0.020 ml was placed in a cylindrical quartz sample tube which holds the sample rigidly in the center of the electron paramagnetic resonance cavity. The second measurement is made by completely filling the aqueous cell with the same solution. It is assumed in the first measurement that all of the sample is within the small sphere of uniform sensitivity of the cavity. Several height and rotational positions were used to determine their effect on the signal intensity and to ensure optimal sensitivity. To test the effect of the different amount of quartz present in the cavity in the two measurements, two cylindrical quartz sample tubes of different glass thickness were compared. They gave identical results to within 3%. According to the weight of the two tubes one had approximately twice the wall thickness of the other. Also the microwave frequency at resonance was only shifted from 9.528 to 9.525 GHz on changing from the aqueous cell to the tube. This is taken to indicate that the sensitivity of the instrument is nearly identical for the two systems. Changing the solvent in the aqueous cell from water to benzene also had only a minor effect on the frequency of resonance. This determination of the effective volume takes into account variations in the cavity sensitivity with vertical and horizontal distance.[3]

Results and Discussion

Using suitable instrumental conditions (see footnote *a* of Table II), the concentration of the component giving rise to the *R. rubrum* electron paramagnetic resonance photosignal was compared (Table II) with the concentration of $P_{0.44}$ measured by change in absorbance at 865 nm. It can be seen that, within the precision of the experiment, the two concentrations are identical.

Similar data are also shown in Table II for chromatophores from *R. spheroides*. Again, the agreement is quite good.

[3] J. Hyde, personal communication, 1968; Varian E-3 Manual Publication No. 87 118-001, 4.2.5.

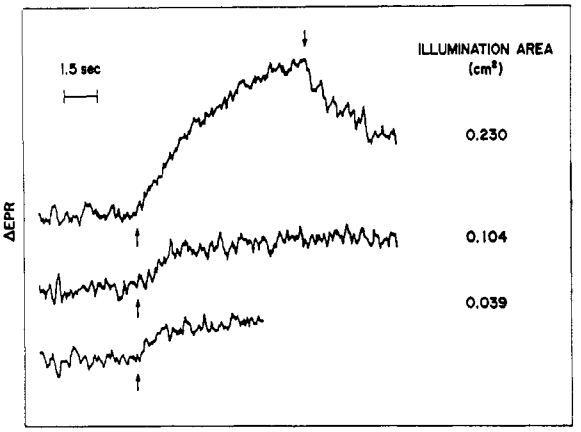

FIGURE 4: Rate of electron paramagnetic resonance signal formation in chromatophores of *R. rubrum*. A_{880} = 100 in a 1-cm cuvet; 0.1 M glycylglycine buffer (pH 7.5).

Measurements of the rate of change in the electron paramagnetic resonance photosignal were made under a variety of conditions of light intensity and illumination area. Representative sets of data are shown in Figure 4. Using the average initial rate of change measured from many separate experiments performed under similar conditions, the quantum yields shown in Table III were calculated. No significant variation in the rate of electron paramagnetic resonance signal production was observed with different chromatophore preparations. Care had to be taken so that the room lights were carefully shielded from the sample as, even when the sample was in the E-3 cavity, some 20–30% of the reaction center $P_{0.44}$ could be in the oxidized state. Also, 0.01 M $K_4Fe(CN)_6$ was present as a redox buffer to keep the trap fully functional. We have observed that in some preparations, in the absence of added redox buffers, some of the reaction center $P_{0.44}$ may be in the oxidized state. Quantum yield measurements with such a system would give low results.

The value for the quantum yield shown in Table III for the photoproduction of free radicals is clearly close to one. This is in excellent agreement with the determination of the quantum yield for $P_{0.44}$ photooxidation from ΔA data (0.95 ± 0.05 (Loach and Sekura, 1968)). In addition, the total quantity of $P_{0.44}$ present is the same whether measured by electron paramagnetic resonance or absorbance changes (Table II).

Consideration of these results along with the shape of the signal (Calvin, 1959; Mauzerall, 1968; Mauzerall and Feher, 1964; Mauzerall *et al.*, 1967), the ability to form it at low temperature (Androes *et al.*, 1962), its redox midpoint potential (Loach *et al.*, 1963), and its dark decay rate (Loach and Sekura, 1967) make it an inescapable conclusion that the photoproduced electron paramagnetic resonance signal and the photoproduced absorbance changes at 865 nm in *R. rubrum* are two physical parameters measuring the same chemical event; that is, an oxidation of a special bacteriochlorophyll molecule which serves as the primary electron donor in the phototrap.

Unfortunately, no well-established actinometer system exists which is characterized by a simple electron paramagnetic resonance signal. In fact, we are not aware of any previous quantum yield determinations conducted using electron paramagnetic resonance techniques. Therefore, it has not been possible to determine, by electron paramagnetic resonance, the quantum yield of an unknown system by a comparison method. On the basis of the results reported herein, we would like to suggest that an appropriate photosynthetic system, such as well-washed and fresh chromatophores from a pure culture of *R. rubrum*, constitutes one of the best actinometer systems that can be used in either visible spectroscopy or electron paramagnetic resonance spectroscopy. Distinct advantages of these photosynthetic systems include a wide choice of exciting wavelengths and a quantum yield very near the theoretical value of 1.00 for many of the longer wavelengths.

TABLE III: Quantum Yields for $P_{0.44}$ Photoxidation in Chromatophores.

Type of Measurement	*R. rubrum*	*R. spheroides*
A_{865} (ϵ_{mM} 90)	0.95 ± 0.05[a]	0.95 ± 0.10[b]
Electron paramagnetic resonance	0.8 ± 0.2	0.7 ± 0.2

[a] Loach and Sekura (1968). [b] Bolton *et al.* (1969).

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The Biochemistry of Long-Chain, Nonisoprenoid Hydrocarbons. III. The Metabolic Relationship of Long-Chain Fatty Acids and Hydrocarbons and Other Aspects of Hydrocarbon Metabolism in *Sarcina lutea**

Phillip W. Albro† and John C. Dittmer

ABSTRACT: Fatty acids added to cultures of *Sarcina lutea* caused changes in the hydrocarbon composition of the cells.

These changes were consistent with a mechanism of synthesis in which the major fatty acid of the cells condensed with the added fatty acid or with fatty acids that increased in response to the added fatty acid and in which one of the fatty acids participating in the condensation was decarboxylated. In the presence of acetate in the medium, exogenous palmitate was incorporated into the hydrocarbon by a mode of entry in which it was specifically not decarboxylated. In media with low acetate, 60–70% of exogenous palmitate incorporated into the hydrocarbon was decarboxylated. Under conditions of incorporation in which the palmitate was not decarboxylated, the carboxyl carbon of the palmitate occurred in monounsaturated hydrocarbons specifically on the side of the double bond opposite that in which the remainder of the aliphatic chain from palmitate was located. Evidence for the direct conversion of monounsaturated hydrocarbons into saturated derivatives and for the failure of ketones to serve as intermediates in the incorporation of fatty acids into hydrocarbons is presented. Alternative mechanisms for the intermediary conversion of fatty acids into hydrocarbons by the condensation of the carboxyl carbon and α-carbon of acids with decarboxylation of one of the acids (head-to-head condensation) that bypasses the requirement for ketones or secondary alcohols are presented.

The large proportion of monounsaturated hydrocarbons in *Sarcina lutea* that have branched methyl groups at both ends of the molecule and the high proportion of branched-chain fatty acids in the lipids of this organism suggested that the hydrocarbons were synthesized by head-to-head condensation of two molecules of fatty acids. Oxidation of the double bond of the C-29 monounsaturated hydrocarbons gave rise to equal amounts of *anteiso*-C-15 and -C-14 fatty acids (each approximately 41% of the total), and this coupled with the fact that no *anteiso*-C-14 fatty acid occurred in the cell lipids further suggested that decarboxylation of one of the fatty acids occurred in the condensation mechanism (Albro and Dittmer, 1969a). Subsequent studies on the incorporation of the ^{14}C-labeled aliphatic chains of isoleucine and valine and of acetate into fatty acids and hydrocarbons of *S. lutea* were fully consistent with this mechanism for hydrocarbon biosynthesis (Albro and Dittmer, 1969b). We present here data from *in vivo* studies on the incorporation of ^{14}C-labeled fatty acids into hydrocarbons that further support the fatty acid head-to-head condensation mechanism and give some insight into the details of the mechanism. Preliminary studies of the role of aliphatic ketones in the biosynthesis and the interconversion of alkenes and alkanes are also reported.

Methods

General culture conditions for *S. lutea*, isolation procedures for lipids, details of gas–liquid partition and thin-layer chromatographic systems, and methods used for assaying radioactivity in lipid samples are described in the first two papers of this series (Albro and Dittmer, 1969a,b). Palmitic-16-^{14}C and -1-^{14}C acids (Calbiochem) with radiopurities of 98%, myristic-1-^{14}C

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